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Cloning of P8, A Transcription Factor Required of Skeletogenic Gene Expression

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**6. AUTHOR(S)**

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**13. ABSTRACT** *(Maximum 200 words)*

The transcription factor toward which this project was directed (formerly known as P8, now as Sp(G/C)F-1) interacts with regulatory regions of many sea urchin embryo genes, and probably acts as a general enhancer protein. During this year this factor was: *i*, purified by affinity chromatography; *ii*, microsequence was obtained; *iii*, the factor was sequenced; *iv*, its DNA binding site was determined; *v*, it was expressed in bacteria and an antibody generated; *vi*, its provenance was determined; and an unusual aspect of its structure was analyzed. The latter is that the factor appears in sea urchin embryo nuclear extract in five different forms of decreasing molecular weight. These forms are also presented in cell-free translation using synthetic mRNA from the cloned cDNA. We shoed that—unusually—there are five successive ATG start sites in this mRNA, all used more or less equally, thus accounting for five nested forms. The sequence reveals Sp(G/C)F-1 to be a novel DNA-binding protein, unlike any in the data banks. It is loaded into the egg in relatively large quantities during oogenesis and then appears in embryo nuclei.

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# FINAL REPORT

Grant #: N00014-93-1-1400

PRINCIPAL INVESTIGATOR: Eric H. Davidson

INSTITUTION: California Institute of Technology

GRANT TITLE: Cloning of P8, A Transcription Factor Required for Skeletogenic Gene Expression

AWARD PERIOD: 9/1/93 - 8/31/96

OBJECTIVE: This is an AASERT Graduate Training Project, the object of which is to isolate, purify, clone, and characterize transcription factors that interact with the regulatory domains of skeletogenic genes in the sea urchin.

APPROACH: The known target site of the factor Sp(G/C)F-1, which interacts with the *SM50* and *SM30* skeletogenic genes, provides the means to purify this protein, clone it and characterize its interaction with DNA, its own structure, its provenance, and its particular function.

ACCOMPLISHMENTS: The transcription factor toward which this project was directed (formerly known as P8, now as Sp(G/C)F-1) interacts with regulatory regions of many sea urchin embryo genes, and probably acts as a general enhancer protein. During this year this factor was: *i*, purified by affinity chromatography; *ii*, microsequence was obtained; *iii*, the factor was sequenced; *iv*, its DNA binding site was determined; *v*, it was expressed in bacteria and an antibody generated; *vi*, its provenance was determined; and an unusual aspect of its structure was analyzed. The latter is that the factor appears in sea urchin embryo nuclear extract in five different forms of decreasing molecular weight. These forms are also presented in cell-free translation using synthetic mRNA from the cloned cDNA. We shoed that—unusually—there are five successive ATG start sites in this mRNA, all used more or less equally, thus accounting for five nested forms. The sequence reveals Sp(G/C)F-1 to be a novel DNA-binding protein, unlike any in the data banks. It is loaded into the egg in relatively large quantities during oogenesis and then appears in embryo nuclei.

SIGNIFICANCE: In the course of this project the Graduate Fellow carrying out the work acquired professional expertise in the complete range of molecular technologies required for transcription factor isolation and characterization. As a result we now have our hands on another of the regulatory molecules that control gene expression, including skeletogenic gene expression.

PATENT INFORMATION: None

AWARD INFORMATION: Received Ph.D.

PUBLICATIONS:

Zeller, R. W., Coffman, J. A., Harrington, M. G., Britten, R. J. and Davidson, E. H. SpGCF1, a sea urchin embryo transcription factor, exists as five nested variants encoded by a single mRNA. *Dev. Biol.* **169**, 713-727, 1995.

Zeller, R. W., Britten, R. J. and Davidson, E. H. Developmental utilization of SpP3A1 and SpP3A2: Two proteins which recognize the same DNA target site in several sea urchin gene regulatory regions. *Dev. Biol.* **170**, 75-82, 1995.

Zeller, R. W., Griffith, J. D., Moore, J. G., Kirchhamer, C. V., Britten, R. J. and Davidson, E. H. A multimerizing transcription factor of sea urchin embryos capable of looping DNA. *Proc. Natl. Acad. Sci. USA* **92**, 2989-2993, 1995.